

# Invitro Anti-Inflammatory Activity and Qualitative Bio-Chemical Analysis of Polyherbal Siddha Formulation - *Vaathathukku Chooranam*

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**Abstract:** Siddha system was propounded by the siddhars and which a vast and unique system which defines Health as a perfect state of physical, psychosocial, social and spiritual wellbeing of an individual. In siddha system, siddhars classified diseases into 4448. Yugimuni mentioned 80 types of vaatha diseases in his text, Yugi vaidhiya chinthamani. In which "Uthiravaatha Sronitham" is one among them and the signs and symptoms of this disease is correlated with Rheumatoid arthritis. Siddha system effectively treats chronic diseases like rheumatoid arthritis, diabetes etc. Traditional siddha medicines offer a wide range of Anti-inflammatory drugs which are polyherbal in nature. This research paper deals with Anti-inflammatory activity and bio-chemical analysis of Vaathathukku Chooranam documented in classical siddha text Agathiyar maruthuvam indicated for Vaatham (Arthritis). This study confirms the Vaathathukku Chooranam possess Anti-inflammatory activity.

**Keywords:** Rheumatoid arthritis, Uthiravaatha sronitham, Vaathathukku Chooranam, Anti-inflammatory activity

## 1. Introduction

Rheumatoid arthritis is a chronic, symmetric, inflammatory peripheral polyarthritis of unknown aetiology. The prevalence of Rheumatoid arthritis is approximately 0.8 - 1.0 % in Europe and Indian subcontinent. The incidence of Rheumatoid arthritis is relatively three times higher in females as compared to the males. It affects approximately 0.5% of the adult population worldwide and occurs 20-50 cases per 1,00,000 annually, mainly in women after their 40's. Rheumatoid arthritis is associated with a variety of extra articular co-morbidities, including cardiovascular disease, resulting in increased mortality in patients with Rheumatoid arthritis. The usage of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of painful musculoskeletal conditions often results in adverse effects such as skin rashes, gastric irritation etc. By the way, polyherbal medicines are potent, efficient, time-tested and devoid of severe side-effects. Hence this current study was

carried out to prove the Anti-inflammatory activity of *Vaathathukku Chooranam* by invitro assays and bio-chemical analysis.

## 2. Materials and Methods

### Drug Selection

The siddha formulation *Vaathathukku Chooranam* taken from the Agathiyar maruthuvam and it is indicated for Vaatham (Arthritis).

Ingredients of *Vaathathukku Chooranam*:

- 1) *Seeragam* ( *Cuminum cyminum* )
- 2) *Karunjeeragam* ( *Nigella sativa* )
- 3) *Sathakuppai* ( *Anethum graveolens* )
- 4) *Chukku* ( *Zingiber officinalis* )
- 5) *Milagu* ( *Piper nigrum* )
- 6) *Thippli* ( *Piper longum* )
- 7) *Kodiveli* ( *Plumbago indica* )

**Table 1:** Information about the Ingredients of *Vaathathukku Chooranam*

S. No	Common name Tamil/ English	Botanical name / Family	Phytochemistry	Actions	Uses in siddha
1.	Seeragam/ Cumin seeds	<i>Cuminum cyminum</i> / Apiaceae	Cuminaldehyde, Cuminal, Cymene, Cuminoside A, B, anisaldehyde, β-pinenine, Cimonene, Eugenol, α-pinene, α-phyllandrones, β-phyllandrones	Anti-inflammatory Anticancer Antibiotic Antimicrobial Antipyretic Antidiarrheal	Piththa disease, Liver disease, Renal calculi, Jaundice, gastric ulcer
2.	Karunjeeragam/ Black cumin	<i>Nigella sativa</i> / Ranunculaceae	Thymol, Thymoquinone, Melanthin, Metarbin, Glucoside, Longitolene, Tetradecanoic acid, Eurucicacid, Campesterol, Glycanol I-palmitate, Buteyl catechol	Antioxidant Antipyretic Anti-infective Anti- diabetic Anti- allergic Diuretic Anthelmintic	Scabies, Jaundice, Ulcer, Eczema, Gastritis, Abdominal bloating
3.	Sathakuppai/Dill	<i>Anethum graveolens</i> / Apiaceae	Carvone, Limonene, Cineole, α-	Anti-inflammatory	Vaatha disease, headache,

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		Apiaceae	phyllandrene, Myrcene, Myristin, Dillapiole	Anti-microbial Analgesic	Ear disease, Kapha disease, Fever, Piles, Chronic sinusitis
4.	Chukku/ Dried ginger	<i>Zingiber officinalis</i> / Zingiberaceae	Zingiberene, Curcumene, Zingerone, Gingerdoils, Gingerols, D-camphor, Zingiberols, Farnesene	Anti-inflammatory Anti-pyretic Anti-oxidant Anti-cancer Anti-infective Anti-ulcer	Indigestion, Ulcer, Headache, Belching, Anorexia, cough
5.	Milagu/Black pepper	<i>Piper nigrum</i> / Piperaceae	Piperine, Piperidine, piperetine, Chavicine, Phenolic amino acids, Alpha tocopherol, Feruperine, Butylated hydroxytoluene, Butylated hydroxyanisole	Anti-tumour Anti-oxidant Anti-bacterial Anti-asthmatic Anti-hypertensive Anti-platelet aggregation	Vaatha disease, Piththa disease, Indigestion, Fever, Ageusia, Kazhalai, Thimir vaatham
6.	Thippili/ Long pepper	<i>Piper longum</i> / Piperaceae	Piperine, Pipertine, Piper longumine, Piper longuminine, Piperide, Piperidine, Asarinine, Pellitorine	Anti-oxidant Anti-cancer Anti-ulcer Anti-microbial Anti-coagulant	Ageusia, Asthma, Cough, Kapha disease, ENT disease, Gastric ulcer
7.	Kodiveli/ Indian leadwort	<i>Plumbago indica</i> / Plumbaginaceae	Plumbagin, Sitosterol, Stigmasterol, $\beta$ -Ayrin, $\alpha$ - sitosterol, 4-hapthoguinone, 5,6 dihydroxy-2-methyl-1	Anti-cancer Anti-bacterial Anti-oxidant Anti-microbial Anti-inflammatory Anti-convulsant	Vaatha disease, Kapha disease, Anemia, Dysentry, Piles, Peptic ulcers

**Collection of Raw Drugs:**

The drugs are purchased from ASN herbal drug shop, Melapalayam, Tirunelveli.

**Authentication of Raw Drugs**

The identification of polyherbal drugs are authenticated by faculties of Department of Gunapadam, Government siddha medical college and Hospital, Palayamkottai.

**Method of Purification**

All the raw drugs are purified as per the methods mentioned in Siddha literature.

**Method of Drug Preparation:**

Vaathathukku Chooranam was prepared according to the procedure mentioned in Siddha classical text Agathiyar maruthuvam.



Figure 1: Vaathathukku Chooranam

**Anti-Inflammatory Activity:**

**Albumin Denaturation Assay Procedure:**

In-vitro anti-inflammatory activity VAC as studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution)

and test sample chloroform extract of VAC at varying concentration ranges from 100 to 500  $\mu$ g/ml along with standard Diclofenac sodium at the concentration of 100  $\mu$ g/ml of final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate.

The Percentage protection from denaturation is calculated by using the formulae

$$\left[ \frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100.$$

**Biochemical Analysis:**

**Preparation of the extract:**

5 grams of the drug was weighed accurately and placed in a 250 ml clean beaker, then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and then it is made to 100 ml with distilled water. This fluid is taken for analysis.

**3. Results and Discussion**

**Anti-Inflammatory Activity:**

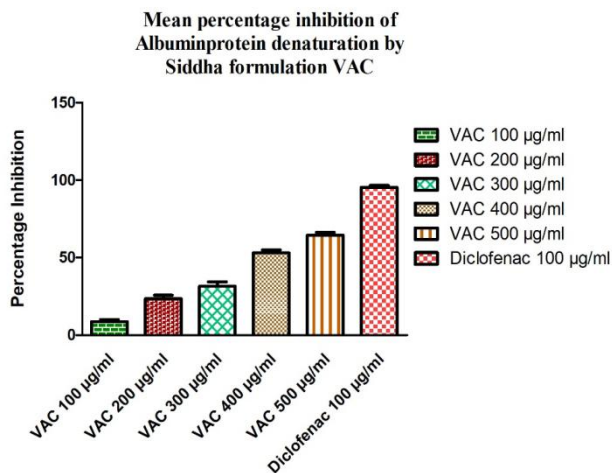
Results are expressed as Mean  $\pm$  SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test.

**Table 2:** Invitro Anti-inflammatory activity of *Vaathathukku Chooranam*

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
VAC 100	8.674 ± 1.376
VAC 200	23.64 ± 2.248
VAC 300	31.55 ± 2.84
VAC 400	53.01 ± 2.055
VAC 500	64.47 ± 1.781
Diclofenac sodium (100 µg)	95.39 ± 1.226

Each value represents the mean ± SD. N=3

**Percentage Inhibition of Protein Denaturation by VAC and Standard**



VAC\*-*Vaathathukku Chooranam*

**Figure 2:** Mean Percentage inhibition of Albuminprotein denaturation by Siddha formulation VAC

The result obtained from the present clearly indicates that the test drug VAC was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 64.47 ± 1.781 % was observed at 500µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 95.39 ± 1.226 at the concentration of 100 µg/ml.

*Vaathathukku Chooranam* is the combination of most efficacious ingredients. While evaluating the ingredients *seeragam*, *sathakuppai*, *chukku*, *kodiveli* and are all exhibits anti-inflammatory property. Along with this, *seeragam* possess Anti-coagulant activity and *sathakuppai* have a Analgesic activity which aids for subsiding inflammatory signs.

**Table 3:** Test Ffor Acidic Radicals

S. No	Procedure	Observation	Inference
1.	<b>Test for calcium:</b> To 2 ml of the above prepared extract taken in a clean test tube. To this add 2 ml of 4% ammonium oxalate solution.	White precipitate is formed	<b>Presence of calcium</b>
2.	<b>Test for sulphate:</b> To 2ml of the extract is added to 5% barium chloride solution.	White precipitate is formed	<b>Presence of sulphate</b>
3.	<b>Test for chloride:</b> The extract is treated with silver nitrate solution.	White precipitate is formed	<b>Presence of chloride</b>
4.	<b>Test for carbonate:</b> The substance is treated with concentrated HCL.	No brisk effervescence is formed	Absence of carbonate
5.	<b>Test for Starch:</b> The extract is added with weak iodine solution.	Blue colour is formed	<b>Presence of starch</b>
6.	<b>Test for ferric iron:</b> The extract is acidified with glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric iron
7.	<b>Test for ferrous iron:</b> The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	Blue colour is formed	<b>Presence of ferrous iron</b>
8.	<b>Test for phosphate:</b> The extract is treated with ammonium molybdate and concentrated nitric acid.	Yellow precipitate is formed	<b>Presence of phosphate</b>
9.	<b>Test for albumin:</b> The extract is treated with esbach’s reagent.	No yellow precipitate is formed	Absence of albumin
10.	<b>Test for tannic acid:</b> The extract is treated with ferric chloride.	No blueblack precipitate is formed	Absence of tannic acid
11.	<b>Test for unsaturation:</b> Potassium permanganate solution is added to the extract.	It gets decolourised	<b>Presence of unsaturated compound</b>
12.	<b>Test for the reducing sugar:</b> To 5 ml of benedict’s qualitative solution is taken in a	Colour changes occurs	<b>Presence of reducing sugar</b>

	test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.		
13.	<b>Test for amino acid:</b> One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.	Violet colour is formed	<b>Presence of amino acid</b>
14.	<b>Test for zinc:</b> The extract is treated with Potassium Ferro cyanide.	No white precipitate is formed	Absence of zinc

The qualitative bio chemical analysis of *Vaathathukku Chooranam* reveals the presence of Calcium, Sulphate, Chloride, Starch, Ferrous iron, Phosphate, Unsaturation compound, Reducing sugar, Amino acid.

This study reveals the presence of Calcium which is very essential constituent of bones, Muscle contraction and nerve transmission and phosphate also have a constituent of bone and teeth. The presence of chloride which is involved in the regulation of acid-base equilibrium. It also ensures that presence of ferrous iron, that helps to increase in the formation of new blood cells which always affected in inflammatory conditions.

#### 4. Conclusion

From this study, we can conclude that the polyherbal formulation of *Vaathathukku Chooranam* possess significant anti-inflammatory property and it contains phytochemicals, so it is the most promising drug for Rheumatoid arthritis. In future in-vivo study will be done on *Vaathathukku Chooranam* for further extensive research.

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